Formulation and evaluation of magnetic microspheres of Cytarabine

A Synopsis

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Introduction

The oral route is the one, most frequently used for drug administration. Oral dosage forms are usually indicated for systemic effects resulting from drug absorption through various epithelia and mucosa of the gastro intestinal tract. Compared with other routes, the oral route is the simplest, most convenient and safest means of drug administration [1]. However oral administration of most of the drugs in conventional dosage forms has short-term limitations due to their inability to restrain and localize the system at gastro-intestinal tract. Microspheres constitute an important part of these particulate drug delivery systems by virtue of their small size and efficient carrier capacity.

Microspheres are the carrier linked drug delivery system in which particle size is ranges from 1-1000 μm range in diameter having a core of drug and entirely outer layers of polymers as coating material [2]

Recently, Novel drug delivery system aims to deliver the drug at a rate directed by the needs of the body during the period of treatment, and target the active entity to the site of action. A number of novel drug delivery systems have emerged encompassing various routes of administration, to achieve controlled and targeted drug delivery, magnetic micro carriers being one of them. Magnetic microspheres are supramolecular particles that are small enough to circulate through capillaries without producing embolic occlusion (<4 μm) but are sufficiently susceptible (ferromagnetic) to be captured in microvessels and dragged in to the adjacent tissues by magnetic fields of 0.5-0.8 Tesla (T).Magnetic microspheres were prepared by mainly two methods namely phase separation emulsion polymerization(PSEP)and continuous solvent evaporation (CSE). The amount and rate of drug delivery via magnetic responsive microspheres can be regulated by varying size of microspheres, drug content, magnetite content, hydration state and drug release characteristic of carrier, the amount of drug and magnetite content of microspheres needs to be delicately balanced in order to design an efficient therapeutic system. magnetic microsphere are characterized for different attributes such as particle size analysis including size distribution, surface topography, and texture etc. using scanning electron microscopy (SEM), drug entrapment efficiency, percent magnetite content, and in vitro magnetic responsiveness and drug release. Targeting by magnetic microspheres i.e. incorporation of magnetic particles in to drug carriers

(polymers) and using an externally applied magnetic field is one way to physically direct this magnetic drug carriers to a desired site. Magnetic microsphere is newer approach in pharmaceutical field. Magnetic microspheres as an alternative to traditional radiation methods which use highly penetrating radiation that is absorbed throughout the body. Its use is limited by toxicity and side effects. The aim of the specific targeting is to enhance the efficiency of drug delivery & at the same time to reduce the toxicity & side effects. This kind of delivery system is very much important which localizes the drug to the disease site [3].

Advantages of Magnetic Microspheres

1. Drug release rates can be tailored to the needs of a specific application; for example, providing a constant rate of delivery or pulsatile release.
2. Controlled release systems provide protection of drugs, especially proteins that are otherwise rapidly destroyed by the body.
3. Controlled release systems can increase patient comfort and compliance by replacing frequent (e.g., daily) doses with infrequent (once per month or less) injection [4].
4. Magnetic microspheres are site specific and by localization of these microspheres in the target area, the problem of their rapid clearance by RES is also surmounted.
5. Linear blood velocity in capillaries is 300 times less as compared to arteries, so much smaller magnetic field is sufficient to retain them in the capillary network of the target area.
6. In case of tumour targeting, microsphere can internalize by tumour cells due to its much increased phagocytic activity as compared to normal cells
7. Problem of drug resistance due to inability of drugs to be transported across the cell membrane can be surmounted [5].

Disadvantages of Magnetic Microspheres

1. By the use of magnetic microspheres in the delivery system, the drug cannot be targeted to deep seated organs in the body.
2. Magnetic targeting is an expensive technical approach and requires specialized manufacturer and quality controlled system.
3. It needs specialized magnet for targeting, advanced technique for monitoring, and trained personnel to perform the procedure [5].

Types of microspheres

- **Bioadhesive Microspheres**

- **Floating Microspheres**
- **Radioactive Microspheres**
- **Polymeric Microspheres**

The different types of polymeric microspheres can be classified as follows and they are biodegradable polymeric microspheres and Synthetic polymeric microspheres.

1. Biodegradable polymeric microspheres
2. Synthetic polymeric Microspheres [6].

- **Magnetic Microspheres**

This kind of delivery system is very much important which localizes the drug to the disease site. In this larger amount of freely circulating drug can be replaced by smaller amount of magnetically targeted drug.

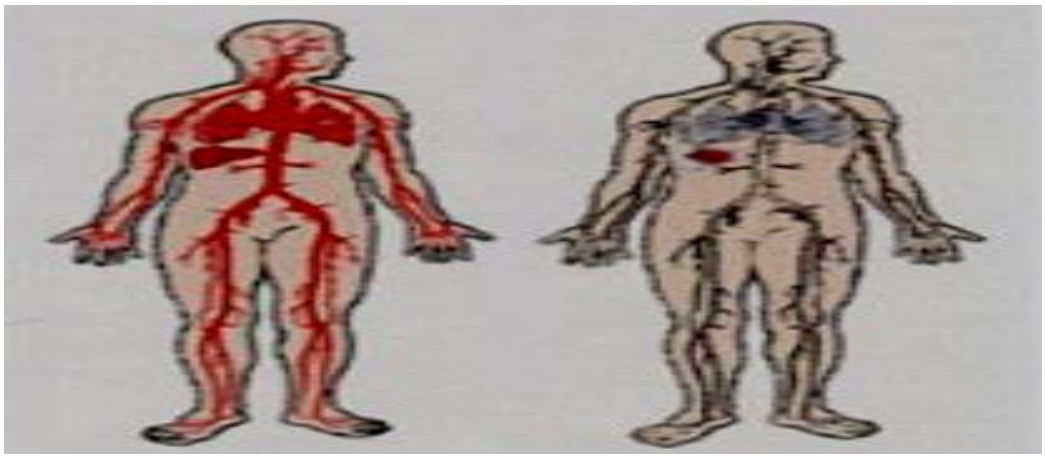
Drugs like proteins and peptides can also be targeted through this system.

Diagnostic microspheres: Can be used for imaging liver metastases and also can be used to distinguish bowel loops from other abdominal structures by forming nano size particles Supramagnetic iron oxides.

Concept of magnetic targeting of microspheres

Ideally, magnetic microspheres are injected into an artery that supplies a given site. As the microspheres would be selectively and magnetically localized at the capillary level, they would have free flow access through the large arteries. Thus the microspheres would serve as time-release capsule system sitting in the desired location. The selective capillary localization of the microspheres can be achieved by taking advantage of the physiological difference in the linear flow velocity of blood at the capillary level (0.05 cm/sec). Obviously, a much lower magnetic field strength is necessary to restrict the microspheres at the slower moving flow velocities of blood in capillaries. After removal of the magnetic field, the microspheres still continued to lodge at the target site, presumable because they had lodged in the vascular endothelium, penetrated in to the interstitial space, resulting in their retention.

The aim of the specific targeting is to enhance the efficiency of drug delivery & at the same time to reduce the toxicity & side effects. Very high concentration of chemotherapeutic agents can be achieved near the target site without any toxic effect to normal surrounding tissue or to whole body [7].



Systemic targeting Magnetic targeting

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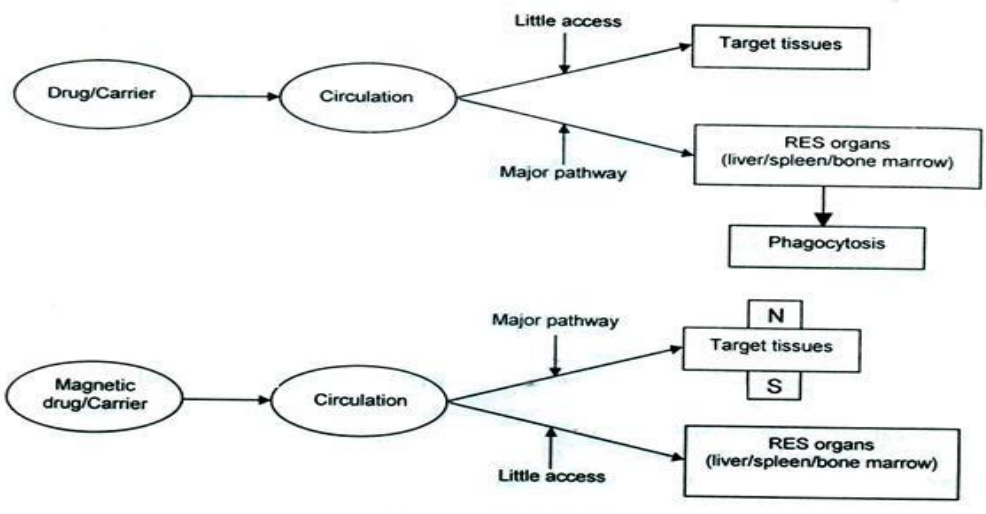
Concept of magnetic targeting by comparing systemic drug delivery with magnetic targeting

Principle of Magnetic Drug Targeting



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Principle of Magnetic Drug Targeting

To improve the efficiency of drug delivery & at the same time to reduce the toxicity & side effects. Cytarabine is formulated as magnetic microspheres

Brief view of Cytarabine

The nucleoside analogue cytarabine (ara-C), an effective and widely used agent for the treatment of acute myelogenous leukemia (AML), induces high initial rates of complete remissions[8].However, only a fraction of these individuals continue to have a long-term disease-free survival[9,10].Accordingly, there is considerable interest in understanding the mechanisms of resistance to ara-C and devising strategies for overcoming them[11].The Chk1 DNA damage kinase is activated by phosphorylation on Ser345 and Ser317 in response to replicative arrest due to the misincorporation of nucleoside analogues into DNA[12,13,14].This event activates an S-phase-specific checkpoint pathway whereby Chk1 inhibits the activity of the Cdc25A phosphatase. In turn, Cdk2 accumulates with elevated levels of phosphorylation on Tyr15 causing cell cycle arrest [15,16]. Thus, the ability of cells to enact a delay in cell cycle progression in response to stalled replication forks and resultant inhibition of DNA synthesis may permit removal of the analogue and repair of damage associated with these processes. In this sense, S-phase arrest may constitute a defense mechanism that spares cells potential toxicity.

Objectives of the study

The objectives of proposed work are:

1. Preformulation studies using drug and excepient.
2. Preparation of Magnetic microspheres using chitosan and sodium alginate as polymers.
3. Evaluation studies of the prepared Magnetic microspheres.

Review of literature

History of magnetic carriers

1. Gilchrist published a seminar paper in 1956 on the selective inductive heating of lymph nodes after injection of 20.100-nm sized magnetite particles into the lymph nodes near surgically removed cancer
2. Turner and Rand combined the radiofrequency heating method with embolization therapy
3. Meyers described how magnetic carriers were able to accumulate small iron particles intravenously injected into the leg veins of dogs, using a large, externally applied horse shoe Magnet. They imagined that it might be useful for lymph node targeting and as a contrast agent
4. Hilal Engineered catheters with magnetic ends, and described how they could be used to deposit and selectively embolize arteriovenous malformations with small magnets.

History of magnetic microspheres

1. Mortazavi *et al* (1996), Microspheres of Albumin were prepared by phase separation method. Dexamethasone was successfully encapsulated into microspheres. Magnesium stearate was used as droplet stabilizer and n- hexane was added to harden the microspheres. The prepared microspheres were characterized for their micromeritic properties and drug loading, as well as FTIR, differential scanning colorimetry, X-ray powder diffractometer, and scanning electron microscopy. The *in vitro* release studies were performed in pH 6.8 phosphate buffer [17].
2. Gadenken *et al* (2001), Protein loaded microspheres were prepared using sonochemical method using Bovine serum albumin. The amount of protein loaded microspheres was determined using spectrophotometer. This method yielded microspheres with a short storage life, low stability, and high toxicity [18].
3. Chatterjee *et al* (2001), Protein loaded polystyrene and albumin microspheres loaded with RBC were prepared by chemical crosslinking method. *In vitro* drug release profiles of polymer-drug were found [19].
4. Emir Baki Denkbas *et al.,* (2002): prepared magnetic chitosan microsphere with a size range of 100-250 mm by the suspension cross linking technique for use in the application of magnetic carrier technology. The magnetic material used in the preparation of magnetic

chitosan microspheres was prepared by precipitation from $FeSO_4$ and $Fe_2(SO_4)_3$ solutions in basic medium and then ground to the desired size (1-5 mm). The morphological and magnetic properties of the microspheres were characterized by different techniques (SEM, Optical microscopy, magnetometry). The results demonstrated that the stirring rate of the suspension medium and Fe_3O_4/Chitosan ratio were the most effective parameters for the size/size distribution and magnetic quality of the microspheres. While the chitosan molecular weight had no effect on these properties for the given molecular weight range (i.e.150 to 650 kDa). The best magnetic quality of the magnetic chitosan microspheres was around 9.1 emu/g microsphere at 10 kG magnetic field intensity [20].

5. Saravanan *et al* (2004), The microspheres of Diclofenac sodium were prepared by emulsification method. The formulated microspheres were characterized by particle size distribution, scanning electron microscopy (SEM), differential scanning calorimetry(DSC), X-ray diffraction and in vitro release studies. The *in vivo* distribution and targetability of gelatin magnetic microspheres after *i.v.* administration were studied in rabbits. Gelatin was used as polymer [21].
6. Zhang *et al* (2007), Composite magnetic Microspheres containing enzymes were prepared by suspension crosslinking technique using chitosan and artemisia seed gum as polymer. Encapsulation efficiency was increased by using a acetic acid glacial solution. The release profile and encapsulation efficiencies depended strongly on the structure of the polymers used as wall materials [22].
7. Yen Fang li *et al,* (2008), The microspheres of enzyme Candida rugosa lipase suspension polymerization of glycidyl methacrylate, ethylene glycol dimethacrylate and vinyl acetate in the presence of oleic acid-coated magnetite, and then, alcoholysed to improve their hydrophilicity [23].
8. Hafeli *et al* (2009), Oleic acid and Polylactic acid coated microcapsules were prepared by emulsion solvent extraction method. The microcapsules were evaluated for size distribution, surface characteristics, drug content, wall thickness and drug release characteristics [24].
9. Jiang *et al* (2011), Carbonaceous polysaccharides loaded microparticles were prepared by low temperature hydrothermal method by using polyacrylic acid. By using this method carbonaceous polysaccharides can be loaded in the microparticles[25].
10. Varma *et al* (2011), The Indomethacin microspheres were prepared and characterized by

incorporation efficiency, particle size, and *in vitro* release behaviour. The drug loaded microspheres showed high incorporation efficiency, scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry(DSC). Sustained release of drug from the microspheres was found by changing drug and polymer compositions. The in vitro drug release mechanism was found. Eudragit was used as a polymer and emulsion solvent evaporation method was used for formation of microspheres [26].

11. Wang *et al* (2012), The controlled release Doxorubicin microcapsules were prepared by water in oil emulsion method using various cellulose polymers. The microcapsules were spherical and free flowing. Doxorubicin (DOX), an anthracycline, is an effective anticancer agent with great antitumor activity against solid tumors and used for the treatment of a number of carcinomas, such as bladder, breast, and gastric cancers. The microcapsules were stable[27].
12. Vyas and Shah (2012), Magnetic microspheres of cisplatin were prepared using phase separation emulsion polymerization technique. Magnetic albumin microspheres formed were retained at their target site in vivo, following the application of magnetic field [28].
13. Kakar and Singh (2014), Magnetic microspheres of mesalamine were prepared by phase separation emulsion polymerization technique. Chitosan microspheres were found to be best among eudragit and ethylcellulose magnetic microspheres[29]
14. Kim *et al* (2015), MRI visible drug eluting magnetic microspheres of amonafide were prepared. This offers the potential for cather-directed drug delivery to liver tumors thereby reducing systemic toxicity and superior therapeutic outcomes [30].

Material and methods

The major areas of the investigation are as follows:

1. Literature survey is conducted to collect a huge literature

2. Preformulation studies will be carried out. The preformulation study will involve the following parameters

- Drug Characterization will be done under the major areas as follows:
  - Drug's Physical appearance will be noted which includes colour, odour of the drug.
  - Melting point of the drug will be determined by capillary method and by DSC.
  - FTIR Spectrum of drug and polymers will be performed to verify whether the drug is compatible with the polymer.
  - Partition coefficient of the drug will be determined.
  - Solubility Profile of the drug will be checked with different solvents.
  - Saturation solubility of drug in various media will be performed.
  - Absorption maxima(λ_{max}) of the drug will be calculated by UV method
- Drug Polymer interaction studies will be performed to check any incompatibility if present between drug and polymer.

3. Calibration curves will be prepared using various solvents

4. Magnetic Microspheres will be prepared

5. Magnetic Microspheres will be characterized as follows:

1. Determination of Percentage Yield (w/w)
2. Determination of Flow Properties
3. Morphology
4. Determination of drug content and drug entrapment efficiency
5. Determination of Magnetite Content:
6. Swelling characteristic of Magnetic Microspheres
7. Magnetic responsivity of Cytarabine loaded Magnetic Microspheres
8. *In-vitro* drug release
9. *In-vitro* drug release kinetics
10. *In vivo* targeting study[31,32]

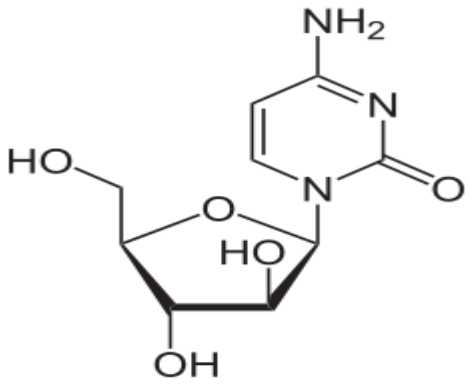
MATERIALS

1. Drug: -Cytarabine

   Drug profile

   Cytarabine

   a)Structural formula:$C_9H_{13}N_3O_5$



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   b) Brand name: **Cytosar U**, **Tarabine PFS**(Pfizer),**Depocyt**(longer-lasting liposomal formulation), **Ara C**

   c) Bioavailability: orally:20%

   d) Metabolism: metabolized in liver

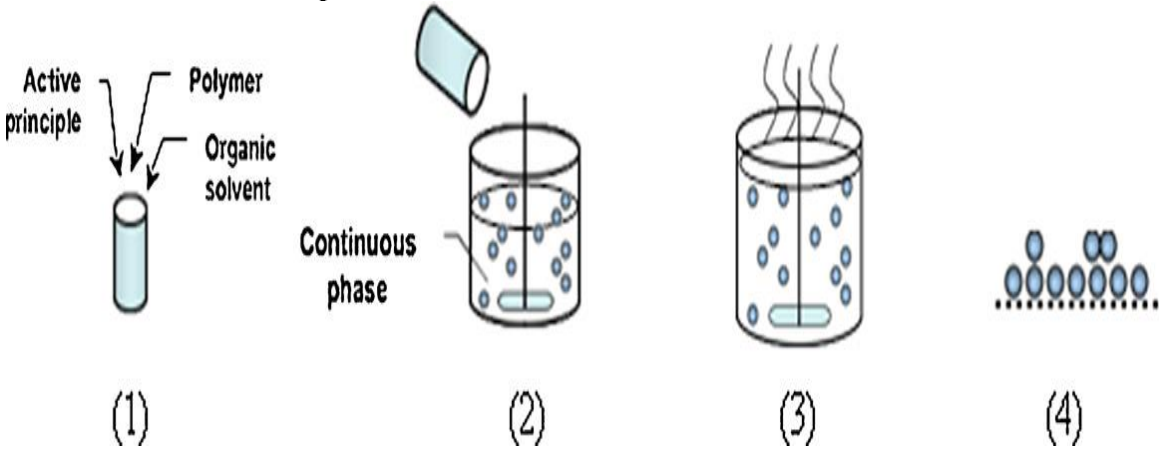
   e)Half-life:1-3 hr

2. Polymers:- Chitosan, sodium alginate
3. Instruments:-UV-visible spectrophotometer, scanning electron microscopy (SEM), FTIR, and Dissolution apparatus.

METHODS

Preparation of microspheres

Continuous solvent evaporation method



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Solution in volatile organic solvent

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Auxillary solution

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Homogenization

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Magnetic microspheres

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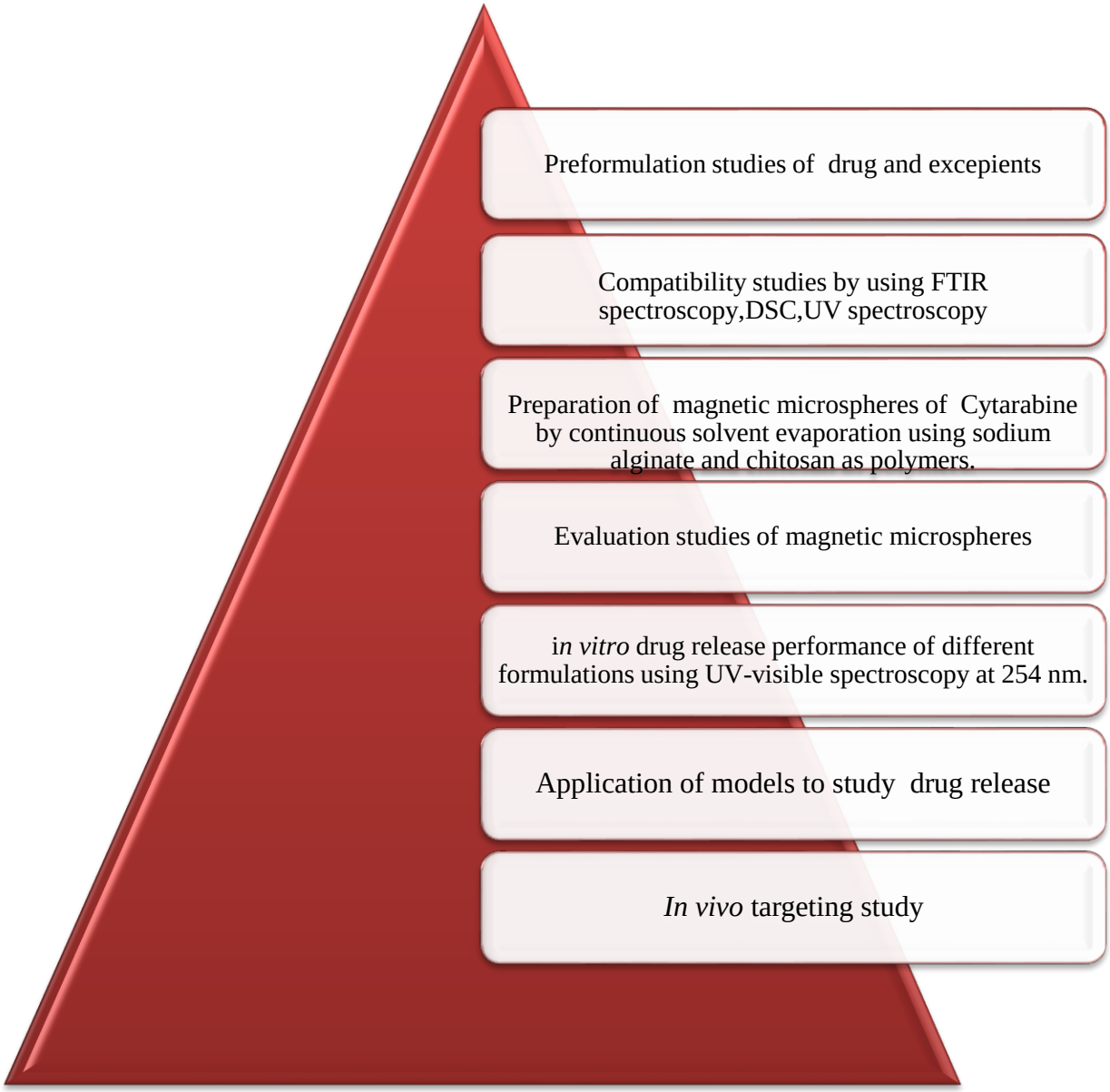
Freeze drying and stored at 4ºC[33]


Preformulation studies of drug and excepients
Compatibility studies by using FTIR spectroscopy,DSC,UV spectroscopy
Preparation of magnetic microspheres of Cytarabine by continuous solvent evaporation using sodium alginate and chitosan as polymers.
Evaluation studies of magnetic microspheres
in *vitro* drug release performance of different formulations using UV-visible spectroscopy at 254 nm.
Application of models to study drug release
In vivo targeting study

References

1. Bhalla. Neetika deep Arsh, Goswami Manish, (2012). "An Overview on various approaches to Oral controlled Drug Delivery System Via Gastroretentive Drug Delivery System", RJP 3 (4):1-6
2. Harshad Parmar, Sunil Bakliwal, Nayan Gujarathi, Bhushan Rane, Sunil Pawar,(2010)."Different Methods of Formulation and Evaluation of Mucoadhesive Microsphere". International Journal of Applied Biology and Pharmaceutical Technology 1(3):1157-1166
3. Swarnima pandey, Sushant kama,(2011)"An overview on multifunctional nanomedicines for targeted drug delivery". JPI's Journal of Pharmaceutics and Cosmetology 2(1):120-128
4. Kyekyoon "Kevin" Kim and Daniel W. Pack University of Illinois at Urbana-Champaign, microspheres for drug delivery ,http://www.springer.com/978-0-387-25563-7
5. Tarun P, Soni S, Thakar B, Pandya V, Bharadia P (2012)."Magnetic Microspheres as a Targeted Drug Delivery System: A Review". International Journal for Pharmaceutical Research Scholars (IJPRS);1(2):444-456
6. Prasanthv.v, Akash Chakraborthy Moy, Sam T Mathew, Rinku Mathapan(2011). "Microspheres - An Overview". International Journal of Research in Pharmaceutical and Biomedical Sciences; 2 (2):332-338.
7. Johansen, L., Nustad, K., Berg Orstavik, T., Ugelstad, J., Berge, A., and Ellingsen, T. (1983).Excess Ab immunoassay for rat glan-965 Mono-sized polymer particles as the prefered solid phase material. J. Immunological Methods; 59: 255-264
8. Cripe LD, Hinton S (2000). "Acute myeloid leukemia in adults". Curr Treat Options Oncol.; 1: 9-17.
9. Schiffer CA (2001) "Acute myeloid leukemia in adults: where do we go from here?"Cancer Chemother Pharmacol; 48(Suppl 1): S45-S52.
10. Tallman MS, Gilliland DG, Rowe JM (2005). "Drug therapy of acute myeloid leukemia" Blood; 106: 1154-1163.
11. Cros E, Jordheim L, Dumontet C, Galmarini CM (2004) "Problems related to resistance to

cytarabine in acute myeloid leukemia". LeukLymphoma; 45: 1123-1132.

12. Zhao H, Piwnica-Worms H. (2001) "ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1". Mol Cell Biol; 21: 4129-4139.
13. Sampath D, Shi Z, Plunkett W. (2002) "Inhibition of cyclin-dependent kinase 2 by the Chk1-Cdc25A pathway during the S-phase checkpoint activated by fludarabine: dysregulation by 7-hydroxystaurosporine". Mol Pharmacol.; 62: 680-688.
14. Shao RG, Cao CX, Pommier Y (2004). "Abrogation of Chk1-mediated S/G2 checkpoint by UCN-01 enhances ara-C-induced cytotoxicity in human colon cancer cells". Acta Pharmacol Sin; 25: 756-762.
15. Mesa RA, Loegering D, Powell HL, *et al* (2005). "Heat shock protein 90 inhibition sensitizes acute myelogenous leukemia cells to cytarabine." Blood; 106: 318-327.
16. Maude SL, Enders GH (2005). "Cdk inhibition in human cells compromises chk1 function and activates a DNA damage response". Cancer Res; 65: 780-786.
17. Sussan Ghassabian,Turaj Ehtetazi, Seyed Mohutan Forutan,Seyed Alireza Mortazavi(1996)" Dexamethasone loaded magnetic albumin microspheres", International journal of pharmaceutics;130:39-55
18. S. Avivi(Levi), I. Felner , I. Novik , A. Gedanken (2001), "The preparation of magnetic proteinaceous microspheres using the sonochemical method",Biochimica et Biophysica Acta;1527:123-129
19. Jhunu Chatterjee, Yousef Haik, Ching-Jen Chen, Modi (2001) "cation and characterization of polystyrene-based magnetic microspheres and comparison with albumin-based magnetic microspheres". Journal of Magnetism and Magnetic Materials ;225:21-29
20. Moroz P,jones SK, Gray BN(2002) "Tumor response to arterials embolization hyperthermia and direct injection hyperthermia in a rabbit liver tumor model". J.surg oncol ;80:149-156
21. Muniyandy Saravanan, Kesavan Bhaskara, Gomathinayagam Maharajan, Kalathil Sadasivan Pillai.(2004) "Ultrasonically controlled release and targeted delivery of diclofenac sodium via gelatin magnetic microspheres", International Journal of Pharmaceutics ;283:71–82
22. Ji Zhang, Shengtang Zhang, Yunpu Wang, Jiayu Zeng, (2007) "Composite magnetic microspheres: Preparation and characterization". Journal of Magnetism and Magnetic Materials ;309:197–201

23. Yang Yong, Yong-Xiao Bai, Yan-Feng Li, Lei Lin, Yan-Jun Cui, Chun-Gu Xia(2008) "Characterization of Candida rugosa lipase immobilized onto magnetic microspheres with hydrophilicity Process" Biochemistry ;43:1179–1185
24. Hong Zhao, Katayoun Saatchi, Urs O. Hafeli (2009) ,"Preparation of biodegradable magnetic microspheres with poly(lactic acid)-coated magnetite", Journal of Magnetism and Magnetic Materials ;321:1356–1363
25. Wei Jiang, Xiaojuan Zhang , Zhendong Sun , Ying Fang , Fengsheng Li , Kai Chen Cheng Huang,(2011)"Preparation and mechanism of magnetic carbonaceous polysaccharide microspheres by low-temperature hydrothermal method", Journal of Magnetism and Magnetic Materials ;323: 2741–2747
26. M Vimal Kumar Varma, P Amareshwar, Raj Kumar Devara,(2011) "Synthesis and Characterization of Magnetic Methyl Methacrylate Microspheres Loaded with Indomethacin by Emulsion Solvent Evaporation Technique" ,International Journal of Drug Delivery;3(1):101-108
27. Ke Wang,Jian Feng Xinga, XingYi Li, Qiang Fua, WeiFeng Li,(2012)"Fabrication of novel magnetic nanoparticles-coated P(styrene-itaconic acid-divinyl benzene)microspheres" Carbohydrate Polymers ;87:2712– 2717
28. Vyas MB, Shah SK(2012) "Design and characterization of cisplatin magnetic microspheres" International Journal for Pharmaceutical Research Scholars,1(4):25-32
29. S Kakar, R Singh(2014) "Preparation of magnetic microspheres of mesalamine by phase separation emulsion polymerization technique" African Journal of Pharmacy and Pharmacology,8(9):246-258
30. Dong Hyun Kim,Jeane Chen, Reed A Omary, Andrew C.Larson (2015) "MRI visible drug eluting magnetic microspheres for transcatheter intra-arterial delivery to liver tumors" Theranostics ;5(5):477-488
31. J.Josephine Leno Jenita,Vijaya K, Suma .Ra, Bincy Raj,and Ayesha Siddiqca"Formulation and Evaluation Of Compression Coated Tablets of Mesalazine For Colon Delivery" International Journal Of Pharmtech Research,2(1):535-541
32. Vartdal, F., Kvalheim, G., Lea, T., Bosnes, V., Gaudernack, G., Ugelstad, J. and Albrechtsen, D. (1987). "Depletion of T-lymphocytes from human bone marrow. Use of magnetic monosized polymer microspheres coated with T-lympho-cyte-specific monoclonal

antibodies". Transplantion 43: 366-371.

33. Yen, S. P. S., Rembaum, A., Molday, R. S. & Dreyer, (1976) W. J. in Emulsion Polymerization. (edsPurma, I. & Gordon, J.); ACS Symposium Series;2(4):236–257 (American Chem. Soc., Washington, DC,1976)